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TITLE: Microenvironment-Programmed Metastatic Prostate Cancer Stem Cells (mPCSCs)

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14. ABSTRACT

Prostate cancer (PCa) metastasis represents the worst outcome that eventually kills the patient. Although many PCa cell-intrinsic molecules and end-organ factors have been implicated in the metastatic dissemination of PCa cells, the role of primary tumor microenvironment and the nature of the metastatic PCa cells remain poorly defined. By establishing a reliable and quantifiable experimental PCa metastasis model in NOD/SCID mice, we have found that PCa cells implanted orthotopically (i.e., in the prostate) metastasize much more extensively and widely than those implanted ectopically (i.e., subcutaneously or s.c). Microarray-based gene expression profiling reveals that the orthotopically implanted human PCa cells upregulate several classes of genes that have been intimately implicated in metastasis. These and many other preliminary observations allow us to HYPOTHESIZE that PCa cells reciprocally interact with the host cells to establish a proinflammatory microenvironment highly conducive to PCa metastasis and that metastatic PCa cells are endowed with CSC properties. By now we have accomplished all goals in Aims 1 and 2. Our lab recently relocated from the MD Anderson Cancer Center to Roswell Park Cancer Institute in Buffalo. We request a 6-month no-cost extension to wrap up a critical set of experiments testing the possibility that HOXB9 regulates PCa metastasis in a context- and model-dependent manner.

15. SUBJECT TERMS

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Department of Defense PCRP IDEA Award
PROGRESS REPORT (Sept 13, 2015 to Sept 12, 2016)

W81XWH-13-1-0352, "Microenvironment-Programmed Metastatic Prostate Cancer Stem Cells (mPCSCs)"

PI: Dean Tang

1. INTRODUCTION: The main goal of this IDEA project is to help elucidate the cellular and molecular mechanisms underlying prostate cancer (PCa) metastasis. Specifically, we test the overarching hypothesis that *prostatic microenvironment facilitates PCa metastasis by promoting the phenotypic as well as functional manifestations of metastatic prostate cancer stem cells (mPCSCs)*. In the application, we proposed three Specific Aims:

1) To perform functional studies on the genes upregulated in the DP human prostate tumors;
2) To test the hypothesis that the DP human PCa cells overexpressing CSC markers possess mPCSC properties; and
3) To test the hypothesis that HOXB9 represents a 'master' regulator of mPCSCs and PCa metastasis.

2. KEYWORDS: Prostate cancer; metastasis; microenvironment; stem cells; cancer stem cells; orthotopic implantation; ectopic implantation; metastatic prostate cancer stem cells

3. ACCOMPLISHMENTS:

Dr. Tang, the PI of this grant, together with most lab members, moved from the M.D Anderson Cancer Center (MDACC) to Roswell Park Cancer Institute (RPCI) on June 1 of 2016. During the period of May 1 – early July of 2016, the lab had been mostly focused on moving related matters and therefore there was a gap in executing experiments related to this project. We have by now finished all goals proposed in Aims 1 & 2 but we still have some work to be accomplished for Specific Aim 3 (see below). Consequently, we request the transfer of the remaining balance for this grant to the RPCI and a 6-month no-cost extension to allow us to complete all goals proposed in Aim 3.

Major Goals of the Project (SOW):

Specific Aim 1: To perform further functional studies on the genes upregulated in the DP human prostate tumors (months 1 – 24).

The main goal of this Aim is to perform systematic knockdown experiments in several PCa models on the following 12 genes, CXCR4, PROM1 (CD133), NOS2A, TACSTD2 (TROP2), LRIG1, ABCG2, CD24, WNT4, ID3, NKX3.1, SMAD1, and HOXB9, and to determine the impact of their knockdown on the metastatic potential of human PCa cells in the mouse DP.

A). Test 3 independent shRNA lentiviral vectors for each gene (i.e., a total of 36 knockdown vectors together with 3 control shRNA lentivectors targeting non-coding scramble, GFP, and luciferase) and determine their knockdown efficiency by performing qPCR and Western blotting analysis.

B). Employ the most efficient vector for each gene (i.e., 12 in total plus control vectors) for in vivo tumor/metastasis experiments first by working on PC3 and xenograft-purified LAPC9 cells.

Specific Aim 2: To test the hypothesis that the DP human PCa cells overexpressing CSC markers possess mPCSC properties (months 12-30)

The main goal of this Aim is to determine whether PCa cells overexpressing CSC surface markers actually possess mPCSC properties, i.e., enhanced metastatic potential.

A*). To determine the metastatic potential of single marker-sorted PCa cells*. (12-24 months).

B*). To determine the metastatic potential of combinatorial marker purified PCa cells.* (20-30 months).

Specific Aim 3: To test the hypothesis that HOXB9 represents a 'master' regulator of mPCSCs and PCa metastasis (months 15-36).

A*). To correlate HOXB9 with PCa progression in patient tumors*. (15-24 months)

B*). To directly determine the functions and mechanisms of HOXB9 in mPCSCs and PCa metastasis*. (20-36 months). We estimate to use ~250 male NOD/SCID mice for these functional studies.

What was accomplished under these goals:

A. *Accomplishment of all goals in Aim 1*.

See last year's Progress Report.

B. *Accomplishment of all goals in Aim 2*.

By now we have completed this Aim. From last year's Progress Report, we proposed to test several combinatorial marker profiles as potentially superior mPCSC markers (to single markers). Our lab has been systematically dissecting PCa cell heterogeneity in the context of their tumor-generating, tumor–propagating, and metastatic potentials (1-9). One strategy we have employed is to frequently compare PCa cell populations bearing double- or triple-marker profiles with those expressing a single marker. For instance, we initially showed that the $CD44^+\alpha2\beta1^+$ PCa cell population in xenograft models is more tumorigenic than either $CD44^+$ or $\alpha2\beta1^+$ cell population (3). Interestingly, we have recently observed that this phenomenon may be model-dependent (6, 9). For example, the $CD44^+\alpha2\beta1^+$ PCa cell population in LAPC9 and LAPC4 xenograft models is more tumorigenic than either $CD44^+$ or $\alpha2\beta1^+$ cell population (6). However, the $CD44^+\alpha2\beta1^+$ PCa cell population in the DU145 model appears to be slightly less tumorigenic than either $CD44^+$ or $\alpha2\beta1^+$ cell population (see Table 1 in ref. 6). This model-dependent difference in tumor-regenerating properties between single-marker vs. combinatorial marker positive populations also seem to apply to their differences in mediating metastasis. Specifically, we have observed that the $CD44^+\alpha2\beta1^+$ or the $CD44^+\alpha2\beta1^+ALDH^{hi}$ (i.e., TM^+; 9) PCa cell population in the LAPC9 but not DU145 model is more metastatic when implanted in the dorsal prostate than single marker-positive cell population. We have just recently obtained this data and are in the process of summarizing the data for publication.

C. *Accomplishment of part of the goals in Aim 3*.

The original goal of Aim 3 is to test the hypothesis that *HOXB9 represents a 'master' regulator of mPCSCs and PCa metastasis via regulating the TGFβ/SMADs signaling which in turn controls CSC molecules such as SPP1, MMP9, CD44, and CD24* (see Figure 5e in the original proposal). Since our last year's Progress Report, Pubmed search on "HOXB9 AND prostate

cancer" still just turns up one reference (10), suggesting that either HOXB9 represents an extremely novel PCa metastasis regulator or HOXB9 might be a context- and model-dependent regulator of PCa metastasis. Our bioinformatics based correlation studies on *HOXB9* mRNAs with various patient parameters revealed, surprisingly, reduced *HOXB9* mRNA levels in prostate tumors vs. normal tissues in TCGA database. Moreover, only a weak upregulation of *HOXB9* mRNA levels is observed in metastatic samples compared with primary tumors in all 9 eligible Oncomine datasets (see Figure 6A in last year's Progress Report). *FINALLY*, Kaplan-Meier survival analysis revealed discordant results that in one data set high *HOXB9* mRNA levels correlated with poor patient survival whereas in two other data sets high *HOXB9* mRNA levels correlated with better overall patient survival (see last year's Progress Report). These observations raise the possibility that ***HOXB9 regulates PCa metastasis in a context- and model-dependent manner***. In the remainder 6 months, we will design several sets of experiments to test this possibility.

References:

1. Patrawala, L. *et al*. Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2^{+} and ABCG2^{-} cancer cells are similarly tumorigenic. *Cancer Res* **65**, 6207-6219 (2005).
2. Patrawala, L. *et al.* Highly purified CD44^{+} prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene* **25**, 1696-1708 (2006).
3. Patrawala, L., Calhoun-Davis, T., Schneider-Broussard, R. & Tang, D.G. Hierarchical organization of prostate cancer cells in xenograft tumors: the CD44$^{+}\alpha2\beta1^{+}$ cell population is enriched in tumor-initiating cells. *Cancer Res* **67**, 6796-6805 (2007).
4. Liu, C. *et al.* The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat Med* **17**, 211-215 (2011).
5. Qin, J. *et al*. The PSA$^{-/lo}$ prostate cancer cell population harbors self-renewing long-term tumor-propagating cells that resist castration. *Cell Stem Cell* **10**, 556-569 (2012).
6. Liu, X. et al. Systematic dissection of phenotypic, functional, and tumorigenic heterogeneity of human prostate cancer cells. *Oncotaget* **6**, 23959-23986 (2015).
7. Rycaj K, et al. Longitudinal tracking of subpopulation dynamics and molecular changes during LNCaP cell castration and identification of inhibitors that could target the PSA$^{-/lo}$ castration-resistant cells. *Oncotarget* 2016 Mar 22;7(12):14220-40. doi: 10.18632/oncotarget.7303.
8. Liu R, et al. miR-199a-3p targets stemness-related and mitogenic signaling pathways to suppress the expansion and tumorigenic capabilities of prostate cancer stem cells. *Oncotarget* 2016 Jul 18. doi: 10.18632/oncotarget.10652. [Epub ahead of print].
9. Chen X, *et al*. Defining a Population of Stem-like Human Prostate Cancer Cells That Can Generate and Propagate Castration-Resistant Prostate Cancer. *Clin Cancer Res*. 2016 Sep 1;22(17):4505-16. doi: 10.1158/1078-0432.CCR-15-2956.
10. De Pinieux, G., *et al.* Clinical and experimental progression of a new model of human prostate cancer and therapeutic approach. *Am J Pathol* **159**, 753-764 (2001).

What opportunities for training and professional development has the project provided?
Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
Aim 1: We have finished Aim 1 and are in the process of summarizing some of the data for a manuscript to be submitted to *Semin. Cancer Biol*. in Dec of this year.

Aim 2: We have finished Aim 2 and are in the process of summarizing some of the data for a manuscript to be submitted to *Semin. Cancer Biol*. in Dec of this year.

Aim 3: In the *final 6-month no-cost extension period*, we design several sets of experiments to test the possibility that *HOXB9 regulates PCa metastasis in a context- and tumor model-dependent manner*. Briefly, we plan to establish Doxycyclin (Dox) inducible HOXB9 expressing LAPC9 and DU145 models and implant these cells subcutaneously, an anatomical site that normally does not support robust systemic metastasis. The expectation is that HOXB9 induction should upregulate various CSC markers, reprogram bulk LAPC9 and/or DU145 cells into mPCSCs and thus facilitate metastasis. In the mean time, we shall also establish Dox-inducible HOXB9 knockdown (KD) LAPC9 and DU145 cells and implant these cells into the DP, a site that supports widespread cancer cell dissemination and metastasis. We predict that HOXB9 KD may suppress LAPC9 and/or DU145 metastasis. We suspect that, based on our earlier tumorigenecity experiments, HOXB9 manipulations may lead to different metastasis phenotypes in LAPC9 vs. DU145 cells.

4. IMPACT:

a. **What was the impact on the development of the principal discipline(s) of the project?**
For the first time, we have generated convincing data that when human PCa cells are implanted subcutaneously in immunodeficient NOD/SCID mice, they readily regenerate tumors but rarely metastasize. In contrast, orthotopically implanted human PCa cells generate less tumors but extensively metastasize. This message should greatly impact how future studies on human PCa metastasis should be modeled and executed.

b. **What was the impact on other disciplines?**
The findings here should also have bearing on similar metastasis studies of other solid tumors such as breast and colon cancers.

c. **What was the impact on technology transfer?**
Nothing to Report

d. **What was the impact on society beyond science and technology?**
Nothing to Report

5. CHANGES/PROBLEMS:
Nothing to Report

6. PRODUCTS:
The current project **intersects** with several other projects in the lab, all of which have a **common** goal, i.e., to dissect PCa cell heterogeneity and to elucidate the role of different

subpopulations of PCa stem/progenitor cells in tumor initiation, maintenance, progression, drug resistance, and metastasis. The following published manuscripts, during the period of Sept 2015 to Sept 2016, have **cited** the partial support of this DOD grant.

1. Gong S, Li Q, Jeter CR, Fan Q, **Tang DG**, Liu B. Regulation of NANOG in cancer cells. *Mol Carcinog*. 2015 Sep;54(9):679-87. doi: 10.1002/mc.22340.
2. Liu X, Chen X, Rycaj K, Chao HP, Deng Q, Jeter C, Liu C, Honorio S, Li H, Davis T, Suraneni M, Laffin B, Qin J, Li Q, Yang T, Whitney P, Shen J, Huang J, **Tang DG**. Systematic dissection of phenotypic, functional, and tumorigenic heterogeneity of human prostate cancer cells. *Oncotarget* 2015 Sep 15;6(27):23959-86.
3. Rycaj K, **Tang DG**. Cell-of-Origin of Cancer versus Cancer Stem Cells: Assays and Interpretations. *Cancer Res*. 2015 Oct 1;75(19):4003-11. doi: 10.1158/0008-5472.CAN-15-0798.
4. Deng Q, **Tang DG**. Androgen receptor and prostate cancer stem cells: biological mechanisms and clinical implications. *Endocr Relat Cancer* 2015 Dec;22(6):T209-20. doi: 10.1530/ERC-15-0217.
5. Zhang D, Park D, Zhong Y, Lu Y, Rycaj K, Gong S, Chen X, Liu X, Chao HP, Whitney P, Calhoun-Davis T, Takata Y, Shen J, Iyer VR, **Tang DG**. Stem cell and neurogenic gene-expression profiles link prostate basal cells to aggressive prostate cancer. *Nat Commun*. 2016 Feb 29;7:10798. doi: 10.1038/ncomms10798.
6. Rycaj K, Cho EJ, Liu X, Chao HP, Liu B, Li Q, Devkota AK, Zhang D, Chen X, Moore J, Dalby KN, **Tang DG**. Longitudinal tracking of subpopulation dynamics and molecular changes during LNCaP cell castration and identification of inhibitors that could target the $PSA^{-/lo}$ castration-resistant cells. *Oncotarget* 2016 Mar 22;7(12):14220-40. doi: 10.18632/oncotarget.7303.
7. Liu R, Liu C, Zhang D, Liu B, Chen X, Rycaj K, Jeter C, Calhoun-Davis T, Li Y, Yang T, Wang J, **Tang DG**. miR-199a-3p targets stemness-related and mitogenic signaling pathways to suppress the expansion and tumorigenic capabilities of prostate cancer stem cells. *Oncotarget* 2016 Jul 18. doi: 10.18632/oncotarget.10652. [Epub ahead of print].
8. Zhang D, Lin K, Lu Y, Rycaj K, Zhong Y, Chao HP, Calhoun-Davis T, Shen J, **Tang DG**. Developing a Novel Two-Dimensional Culture System to Enrich Human Prostate Luminal Progenitors That Can Function as a Cell of Origin for Prostate Cancer. *Stem Cells Transl Med*. 2016 Sep 29. pii: sctm.2016-0243. [Epub ahead of print].
9. Chen X, Li Q, Liu X, Liu C, Liu R, Rycaj K, Zhang D, Liu B, Jeter C, Calhoun-Davis T, Lin K, Lu Y, Chao HP, Shen J, **Tang DG**. Defining a Population of Stem-like Human Prostate Cancer Cells That Can Generate and Propagate Castration-Resistant Prostate Cancer. *Clin Cancer Res*. 2016 Sep 1;22(17):4505-16. doi: 10.1158/1078-0432.CCR-15-2956.
10. Rycaj K, **Tang DG**. Metastasis and metastatic cells: A historical perspective and current analysis. In: Cancer Stem Cells: Targeting the Roots of Cancer, Seeds of Metastasis, and Sources of Therapy Resistance. *Editors*, Huiping Liu & Justin D. Lathia. Academic Press, Elsevier. Pp317-340, 2016.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Ruifang Liu, Ph.D
Project Role:	Postdoc
Researcher Identifier (e.g.	N/A

ORCID ID):	
Nearest person month worked:	9
Contribution to Project:	Dr. Liu was involved in performing some combinatorial marker metastasis assays. She'll also be involved in the HOXB9 metastasis experiments during the proposed 6-month no-cost extension period
Funding Support:	This DOD grant

Name:	Hseuh-Ping (Eva) Chao
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6
Contribution to Project:	Eva was involved in bioinformatically analyzing differentially expressed genes
Funding Support:	This DOD grant

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report

What other organizations were involved as partners?
Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS:
N/A

APPENDICES: